Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology & Therapeutics 105 (2005) 151–163

Pharmacology & Therapeutics

www.elsevier.com/locate/pharmthera

Associate editor: O. Binah

# Cells, scaffolds, and molecules for myocardial tissue engineering

Jonathan Leor[a,*], Yoram Amsalem[a], Smadar Cohen[b]

[a]*Neufeld Cardiac Research Institute, Tel Aviv University, Sheba Medical Center, Tel Hashomer 52621, Israel*
[b]*Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Beer-Sheva, Israel*

**Abstract**

Unlike heart valves or blood vessels, heart muscle has no replacement alternatives. The most challenging goal in the field of cardiovascular tissue engineering is the creation/ regeneration of an engineered heart muscle. Recent advances in methods of stem cell isolation, culture in bioreactors, and the synthesis of bioactive materials promise to create engineered cardiac tissue ex vivo. At the same time, new approaches are conceived that explore ways to induce tissue regeneration after injury. The purpose of our review is to describe the principles, status, and challenges of myocardial tissue engineering with emphasize on the concept of in situ cardiac tissue engineering and regeneration.


*Keywords:* Biomaterial; Cell; Heart; Scaffold; Tissue engineering; Transplantation

*Abbreviations:* 3D, three-dimensional; bFGF, basic fibroblast growth factor; ECM, extracellular matrix; EHT, engineered heart tissue; EPC, endothelial progenitor cell; EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor; LV, left ventricle; MI, myocardial infarction; SDF-1, stromal derived factor; VEGF, vascular endothelial growth factor.

**Contents**



* Corresponding author. Tel.: +972 3 5342278; fax: +972 3 5351139.
*E-mail address:* leorj@post.tau.ac.il (J. Leor).

0163-7258/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2004.10.003

## 1. Introduction

Tissue engineering is a rapidly growing area that aims to create, repair and/or replace tissues and organs by using combinations of cells, biomaterials, and/or biologically active molecules. Tissue engineering strategies promise to revolutionize current therapies for irreversible myocardial damage, heart failure, and significantly improve the quality of life for millions of patients.

The most challenging goal in the field of cardiovascular tissue engineering is the creation of an engineered heart muscle. Today, unlike heart valves or blood vessels, heart muscle has no replacement alternatives. Recent advances in methods of stem cell isolation and culture in bioreactors and the synthesis of bioactive materials show promise to contribute to creation of engineered cardiac tissue in vitro. New discoveries in stem cell biology suggest that stem cells are a potential source of heart muscle cells and blood vessels and can be used by clinicians to rebuild or replace damaged heart tissue.

The purpose of our review is to describe the principles and challenges of myocardial tissue engineering with emphasize on the concept of in situ cardiac tissue engineering.

## 2. Myocardial infarction and repair

### *2.1. Myocardial infarction, ventricular remodeling, and heart failure*

The human heart cannot regenerate significantly because adult cardiac myocytes are terminally differentiated and cannot replicate after injury (Pasumarthi & Field, 2002). Heart failure after MI can result from the substantial loss of cardiomyocytes in the infarct zone but more often is precipitated by the delayed and progressive pathological remodeling of the left ventricle (LV). When myocardial tissue is injured, normal healing response is initiated through a series of complex events that include acute inflammation, the formation of granulation tissue, and eventual scar formation (Sun et al., 2002; Nian et al., 2004). Cytokines and growth factors are released to recruit white blood cells, mainly neutrophils. Monocytes are then called to the wound site where they differentiate into macrophages. The macrophages are responsible for cleaning the infarcted zone and also for recruiting cells such as fibroblasts, endothelial cells, and stem/progenitor cells creating granulation tissue. The formation of blood vessels is essential to the healing of the infarcted myocardium. The granulation tissue is subsequently replaced by an extracellular matrix (ECM) deposited primarily by fibroblasts and the granulation tissue is remodeled into scar tissue.

The problem of how to treat the thousands of patients per year worldwide, who survive an extensive myocardial infarction (MI) and develop advanced heart failure despite optimal medical therapy, has not been resolved (Jessup & Brozena, 2003). Failure to prevent morbidity associated with heart failure places an enormous burden on patients, their families, and the community. Heart transplant is the best solution to patients with end-stage heart failure. However, donor supply is declining, increasing the gap between supply and demand for heart replacement therapies. Left ventricular assist devices may provide a temporary therapeutic option for patients with pump failure, but at best they serve as only a bridge to transplantation and do not provide definitive therapy (Copeland et al., 2004). As a result, there has been great interest in alternative therapeutic strategies to reverse this common and deadly disease.

### *2.2. Myocardial repair*

Cardiac repair is an exciting novel therapeutic concept (Etzion et al., 2001). Through cellular therapies, the concept of "growing" heart muscle and vascular tissue has revolutionized the approach of treating heart disease. In the heart, cellular repair strategies can include:

(1) Direct transplantation of cells into damaged environments.
(2) Tissue engineering techniques for the development of replacement tissue.
(3) Therapies that prompt the heart to regenerate damaged tissues.

The first approach focuses on repopulation of the injured myocardium by transplantation of healthy cells. Several cell types that might replace necrotic tissue and minimize scarring have been considered. Fetal cardiomyocytes, skeletal myoblasts, and bone marrow stem cells have all shown limited success in restoring damaged tissues and improving cardiac function. Failure to produce new myocardial fibers in clinically relevant numbers was attributed to cell death occurring after engraftment and inability of engrafted myoblasts to differentiate and integrate within the host myocardium; hence, electromechanical coupling is not likely to occur after in vivo grafting.

An alternative different approach includes mobilization of progenitor or stem cells to the damaged area or stimulation of a regenerative program within the organ (Minatoguchi et al., 2004). Recent studies have suggested that stem cells reside within the bone marrow or peripheral blood can be recruited to the injured heart (Askari et al., 2003). In addition, there is now accumulating evidence that the heart contains resident stem cells that can be induced to develop into cardiac muscle and vascular tissue (Beltrami et al., 2003; Oh et al., 2003; Matsuura et al., 2004). These cardiac progenitors would be recruited to repair the infarcted myocardium.

## 3. Tissue engineering

### 3.1. Classic paradigm

The goal of tissue engineering is to repair or replace the damaged organ or tissues by delivering functional cells, supporting scaffolds, growth promoting, and signal molecules or DNA encoding these molecules to areas in need. It applies the principles of engineering and the life sciences in an effort to reach a fundamental understanding of structure-function relationships in normal and pathological tissues and to develop biological substitutes that can grow and remodel to restore, maintain, or improve tissue and organ function. The field has already made headway in the synthesis of structural tissues such as skin, cartilage, bone, and bladder (Vacanti & Langer, 1999).

The classic tissue engineering strategy is to isolate specific cells through a biopsy from a patient, to grow them on a three-dimensional (3D) biomimetic scaffold under precisely controlled culture conditions, to deliver the construct to the desired site in the patient's body, and to direct new tissue formation into the scaffold that can be degraded over time (Vacanti & Langer, 1999). In order to achieve successful regeneration of damaged organs or tissues based on the tissue engineering concept, several critical elements should be considered, including the biomaterial scaffold that serves as a mechanical and biological support for cell growth and differentiation, progenitor cells that can be differentiated into specific cell types, and the inductive growth factors that can modulate cellular activities.

Strategies of tissue engineering can be classified as in vitro and in vivo approaches (Fig. 1):

(1) In vitro tissue engineering in culture dish or bioreactor.
   (a) Creation of engineered cardiac graft from cell-seeded scaffold.
   (b) Creation of engineered cardiac graft from cell-seeded biomaterial gel.
   (c) Creation of cell film from cardiac cells and biomaterial sheet.

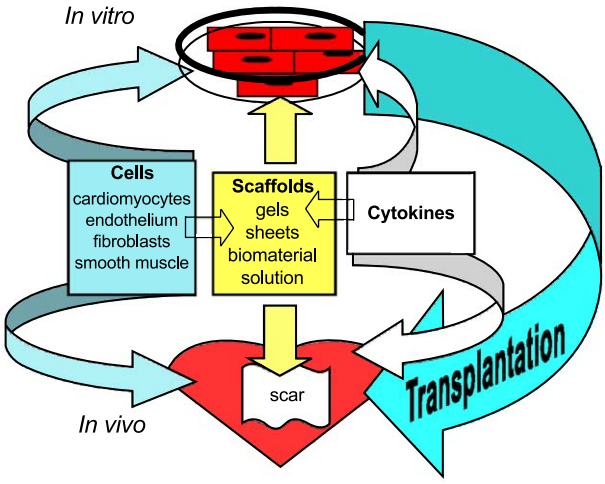


Fig. 1. Strategies of tissue engineering can be classified as in vitro and in vivo approaches. Cells, scaffolds, and cytokines can be used together or independently to engineer replacement myocardial tissue.

(2) In vivo tissue engineering (in situ generation)
   (a) Cell transplantation.
   (b) Cell seeded scaffold implantation.
   (c) Unseeded scaffold implantation and recruiting endogenous cells.
   (d) Injectable scaffold with or without cells.
   (e) Promotion healing and self-repair by delivery of active molecules.

While the in vitro approach provides good control on construct shape, size, cell sources, development, and function, it is limited by the ability to create robust muscle and the risk of tissue necrosis after transplantation. The in vivo approach aims to create replacement tissue in the natural milieu; it is feasible and simpler than the previous one but has been limited by poor control on graft development and outcome.

### 3.2. Cell sources for myocardial tissue engineering

The optimal cell source to create an engineered myocardial patch should be easy to harvest, proliferative, nonimmunogenic, and has the ability to differentiate into mature, functional cardiomyocyte. Unfortunately, no such cell currently exists. Several cell sources have been proposed (Table 1). Donor (allogenic) cells are relatively easier to obtain but entail risky immunesuppression. Autologous cells, on the other hand, are more difficult to obtain and to expand but have no immunologic barriers.

Table 2 describes the advantages and limitations of various cell sources. Theoretically, the natural electrophysiological, structural, and contractile properties of cardiomyocytes make them the ideal donor cell type. However, cardiomyocytes are difficult to obtain, to expand, are sensitive to ischemic insults, and are allogenic, that is, they will evoke immune response in the host tissue.

Table 1
Potential cell sources for myocardial tissue engineering

1. Fetal cardiomyocytes (Li et al., 1999; Leor et al., 2000)
2. Skeletal myoblasts (Kamelger et al., 2004; Li, 2004)
3. Mesenchymal stem cells (Krupnick et al., 2001)
4. Smooth muscle cells (Matsubayashi et al., 2003)
5. Endothelial progenitor cells (Wu et al., 2004)
6. Crude bone marrow (Ryu et al., 2005)
7. Umbilical cord cells (Kadner et al., 2004)
8. Fibroblasts (Li et al., 2000; Kellar et al., 2001)
9. Human embryonic stem cells (Levenberg et al., 2003)
10. Cloned cells (Lanza et al., 2004)

Today, the most widely used cell types for cardiac cell therapy in human patients are skeletal muscle-derived progenitors, or myoblasts, and crude bone marrow mononuclear cells (Lee & Makkar, 2004). Both cell types share advantages over other cells proposed for cardiac repair in that they are readily available, autologous, and easily expanded in vitro. A limitation to the efficacy of myoblasts is their apparent inability for transdifferentiation into cardiac or endothelial cells. In contrast, bone marrow-derived stem cells are currently gaining favor because of their seeming plasticity, which could allow them to alter their phenotype in response to cues from the target organ, and the possibility of using the patient's own cells. A few recent clinical studies advocate the simple extemporaneous reinjection of unfractionated bone marrow cells in patients with acute MI. However, since such studies have been performed at the early stage after the ischemic insult, their relevance to chronically infarcted myocardium remains uncertain.

The use of autologous adult stem cells is particularly restricted by their low recovery from the bone marrow, adipose tissue, or circulation, and therefore, the difficulty in obtaining reasonable numbers of suitable cells (Scheubel et al., 2003). In addition, progenitor cells from sick patients, such as type II diabetics, exhibit impaired proliferation, adhesion, and incorporation into vascular structures (Dimmeler & Vasa-Nicotera, 2003; Rauscher et al., 2003; Scheubel et al., 2003).

Furthermore, safety issues have been raised regarding the use of various cells for myocardial repair: arrhythmias with skeletal myoblasts (Smits et al., 2003), cardiac calcifications with bone-marrow mononuclear cells (Yoon et al., 2004), myocardial scarring with mesenchymal stem cells (Vulliet et al., 2004), and teratoma with human embryonic stem cells (Thomson et al., 1998). In addition, the search continues for an efficient and reproducible method to control and direct differentiation of stem cells to the desired cell type-in vitro (Mummery et al., 2003; Takahashi et al., 2003).

### 3.3. Scaffolds and biomimetic materials

The biomaterial scaffold plays a key role in most tissue engineering strategies. To guide the organization, growth, and differentiation of cells in tissue engineered constructs, the biomaterial scaffold should be able to provide not only a physical support for the cells but also the chemical and biological cues needed in forming functional tissues (Langer & Tirrell, 2004). In essence, the biomaterial should be able to crosstalk, on the molecular level, with the cells in a precise and controlled manner, similarly to the natural interactions existing between cells and the native ECM. At the same time, the basic requirements from a biomaterial should be kept; that is, the materials and their degradation products must be non-toxic and non-immunogenic, and their degradation rate should match the rate of new tissue formation.

Thus, in recent years, the trend has been to design bioactive materials, which on one hand will have the appropriate physical strength as well as the degradation kinetics of synthetic polymers, and on the other hand will have the biological specificity of collagen, fibronectin, and laminin—the major ECM components. Such biological resembling biomaterials, termed "biomimitics", should promote cell-matrix interactions, and elicit specific cellular responses and biomolecular recognition.

The approaches for achieving biomimetic materials include synthesis of new materials from scratch or chemical modification of existing materials with bioactive molecules. The later approach has the advantages of working with known materials, most of which were tested and proved to be safe in human. The bioactive molecules may be whole ECM molecules or "cell-binding" domain sequences isolated from these proteins. The use of short peptides is advantageous over the whole protein because the protein tends to be randomly folded and the receptor binding

Table 2
Advantages and disadvantages of various cell sources for myocardial repair

| | Autologous | Easily obtainable | Highly expandable | Cardiac myogenesis | Clinical experience | Safety concerns |
|---|---|---|---|---|---|---|
| Fetal cardiomyocytes | No | No | No | Yes | No | No |
| Embryonic stem cells | No | No | Yes | Yes | No | Yes teratoma |
| Skeletal myoblasts | Yes | Yes | Depend on age | Debated | Yes | Yes arrhythmias |
| Crude bone-marrow cells | Yes | Yes | Depend on age | Debated | Yes | Yes calcification |
| Mesenchymal stem cells | Yes | No | Depend on age | Yes | No | Yes Fibrosis calcification |
| Hematopoietic stem cells | No | Yes | Yes | Debated | Yes | No |
| Fibroblasts | Yes | Yes | Yes | No | No | No |
| Smooth muscle cells | Yes | Yes | Yes | No | No | No |

domains are not always sterically available. In addition, the short peptide is relatively more stable during the modification process and can be massively synthesized in the lab. The most commonly used peptide sequence derived from fibronectin signaling domain is Arg-Gly-Asp (RGD; Humphries et al., 1986; Griffith & Lopina, 1998; Rowley et al., 1999; Tiwari et al., 2002; Pratt et al., 2004). The selection of peptide sequences for modification depends on the cell type seeded onto the matrix or the implanted site of the scaffold and its natural ECM environment and the specific required cellular responses.

Modification of biomaterial can be performed either in surface or bulk mode. Surface modification of materials with bioactive molecules is a simple way to make biomimetic materials. In most cases, the goal is to study cell attachment, spreading, proliferation, and differentiation on modified surfaces in two-dimensional culture, without addressing the effects of the third dimension. Bulk modification of a material, in comparison, should provide a more suitable environment for the cells as it imitates the 3D environment of the natural ECM. Most of the bulk-modified materials are based on polymers that have been applied before as nonmodified ones for tissue engineering; for example, Hyaluronan (Nguyen et al., 2003), poly(-ethylene oxide) (PEO; Koo et al., 2002), poly(*N*-isopropylacrylamide) (Kim et al., 2002), poly(L-glycolic acid) (PLGA; Mann & West, 2002), and alginate (Rowley et al., 1999). Modification is usually carried out through a chemical reaction leading to covalent bond between the polymer backbone and the bioactive peptide. Another method cross links the polymer to form a hydrogel using bi-functional peptide that also has a signaling domain for interactions with cell membrane receptors (Halstenberg et al., 2002).

The biomimetic materials described herein belong to the third-generation biomaterials, which were designed to enable the cell-matrix cross talk, on the molecular level. It is expected that with a more detailed understanding of cell – matrix interactions and improvement in material design and fabrication, new materials will be evolved that will imitate the physical architecture of natural ECM.

## 4. Current achievements in myocardial tissue engineering

### *4.1. Engineering beating construct in vitro*

Zimmermann et al. (2004) proposed certain criteria for cardiac tissue construct. It should display functional and morphological properties of native heart muscle and remain viable after implantation. Mechanical, electrical, and functional integration into the organ architecture should result in improved systolic and diastolic function of diseased myocardium. Thus, constructs should be (1) contractile, (2) electrophysiologically stable, (3) mechanically robust yet flexible, (4) vascularized or at least quickly vascularized after implantation, and (5) non-immunogenic. Today, such an ideal construct does not exist. However, progress in recent years has led to the development of constructs which fulfill one or more of these ambitious criteria.

A number of groups reported encouraging results with various techniques for constructing cardiac graft for transplantation and showed that neonatal rat or chick embryo cardiomyocytes can be reconstituted to 3D myocardial tissue-like constructs (Akins et al., 1999; Bursac et al., 1999; Carrier et al., 1999; Papadaki et al., 2001b; Carrier et al., 2002; Dar et al., 2002; Shimizu et al., 2002a, 2002b; Zimmermann et al., 2002a, 2002b).

We have shown that cardiomyocyte seeding within porous alginate scaffolds yield 3D high-density cardiac constructs with a uniform cell distribution (Dar et al., 2002). The hydrophilic nature of the alginate scaffold, its >90% porosity and interconnected pore structure, enabled efficient cell seeding onto the scaffold within a short time, up to 30 min. With an aid of a moderate centrifugal force during cell seeding, a uniform cell distribution throughout the alginate scaffolds was achieved, consequently enabling the loading of a large number of cells onto the 3D scaffolds. Some of the cell clusters were contracting spontaneously within the matrix pores. Throughout the culture there was no indication of cardiomyocyte proliferation within the scaffolds, nor was it found in 3D cultures of cardiofibroblasts. This may enable the development of cardiac co-cultures, without domination of cardiofibroblasts with time (Dar et al., 2002).

Bursac et al. (2003) cultured neonatal rat ventricular cells on polymeric scaffolds in bioreactors. After 1 week, all constructs contained a peripheral tissue-like region (50–70 μm thick) in which differentiated cardiac myocytes were organized in multiple layers in a 3D configuration. They proposed such constructs as a model for electrophysiological studies. In a later work, they improved the structural and electrophysiological properties of the engineered constructs by changing parameters of the scaffolds and bioreactors (Bursac et al., 2003). Kofidis et al. (2002, 2003) engineered artificial myocardial tissue samples by seeding neonatal rat cardiomyocytes within commercially available 3D collagen matrix. The artificial myocardial tissues showed continuous, rhythmic, and synchronized contractions for up to 13 weeks. Embedded cardiomyocytes were distributed equally within the 3D matrix. Application of $Ca^{(2+)}$ and epinephrine, as well as electrical stimulation or stretching, resulted in enhanced force development. Electrocardiograph recording was possible on spontaneously beating artificial myocardial tissue samples and revealed physiologic patterns.

As an alternative to seeding the cells on a preformed scaffold, Zimmermann et al. utilized Matrigel or Matrigel mixed with collagen gel (Zimmermann et al., 2000; 2002a, 2002b, 2004; Zimmermann & Eschenhagen, 2003). The cells were mixed with the liquid material which was

solidified by casting in a cylindrical template. After a few days, they moved the tissue patch to a stretching device that simulated the heart's contractions. They demonstrated that collagen type I and ECM proteins when mixed with freshly isolated heart cells join together to a strongly contracting and highly differentiated construct which they name engineered heart tissue (EHT). The geometric shape of EHT could be altered by utilization of suitable casting molds (square, circular). They found that 4 factors are important to reconstitute strongly contracting EHT: (1) addition of Matrigel to the reconstitution mixture (only in rat EHT), (2) EHT culture under mechanical load, (3) a circular shape, in contrast to EHT patches, and (4) utilization of cell mixtures rather than purified cardiac myocyte populations (Zimmermann et al., 2004). Under these conditions, strongly contracting (up to 3 $mN/mm^2$) and morphologically highly differentiated muscle constructs can be engineered (Zimmermann et al., 2002b). Based on this work and others (Akhyari et al., 2002), it seems that cyclical mechanical stretch regimen applied to cardiac cells seeded on a scaffold improves tissue formation and enhance graft strength.

An alternative approach has been proposed by Shimizu et al. (2002b). They grew rat cardiomyocytes on a thin temperature responsive polymer, PIPAAm [poly(*N*-isopropylacrylamide)]. The polymer sheet promoted the thin cell layers to detach when the temperature is reduced, thus releasing cardiac myocyte sheets from the dishes without enzymatic or EDTA treatment. The researchers laid four of these sheets on top of each other until they fused, and the product was implanted under the skin of rats. Six months later, the researchers observed that the engineered cardiac patches were beating and had been infiltrated by blood vessels. One of the potential advantages of this strategy is the ability to stack other necessary cell sheets between cardiomyocyte sheets as endothelial cells in attempt to cope with the perfusion limitation in thick constructs.

Another technique that may accelerate and optimize engineered myocardial assembly is "organ printing" (Mironov et al., 2003). A device which prints gels, single cells and cell aggregates has been developed. Layer-by-layer sequentially placed and solidified thin layers of thermoreversible gel which serve as 'printing paper'. This computer-aided, jet-based 3D tissue-engineering of living human organs suggests a new strategy for growing a patch of cardiac muscle (Mironov et al., 2003). To achieve a better control over the morphology and architecture of cultured cardiomyocytes, McDevitt et al. (2002) laid lanes of laminin, 5–50 μm wide, by microcontact-printing onto non-adhesive (bovine serum albumin [BSA]-coated) surfaces. Adherent cardiomyocytes responded to the spatial constraints by forming elongated, rod-shaped cells whose myofibrils aligned parallel to the laminin lanes. Patterned cardiomyocytes displayed a striking, bipolar localization of the junction molecules *N*-cadherin and connexin43 that ultrastructurally resembled intercalated disks. Similar cardiomyocyte patterns were achieved on micropatterned biodegradable polymer PLGA, suggesting that patterned cardiomyocytes could be used in myocardial tissue engineering (McDevitt et al., 2003).

The results of these pioneering experiments provide a tool to investigate myogenesis and myocardial physiology, biology, and pharmacology ex vivo. In addition, it raises hope for myocardial tissue engineering to repair or replace the infarcted myocardium. Therotically, the bioengineered cardiac tissue could be used for surgical reconstruction of the infarcted myocardium or repair of congenital cardiac defects (Krupnick et al., 2002).

### 4.2. Bioreactors

One of the major difficulties in cardiac tissue engineering is how to grow three-dimensional structures that contain more than a few layers of muscle cells. To improve the results of in vitro tissue engineering, researchers have designed several bioreactors, which portray different patterns of fluid dynamics and vessel geometry. A basic fluid-dynamic cultivation vessel is the Spinner flask, which is an agitated flask usually at 50 rpm (Carrier et al., 1999, 2002). In these vessels, the cell constructs are subjected to turbulently mixed fluid that provides a well-mixed environment around the cell constructs and minimizes the stagnant layer at their surface. It has been shown that cultivation of cardiac cell constructs in Spinner flasks produces engineered tissues that are superior, in almost every aspect (e.g., aerobic cell metabolism, DNA content, metabolic activity, and morphological appearance) to tissues cultivated under static conditions (Carrier et al., 1999, 2002; Papadaki et al., 2001a). The Spinner flask may not, however, be the optimal cultivation vessel for cardiac cells. The turbulent fluid flow at the surface of the constructs is usually characterized by eddies that destroy the seeded cells.

Bioreactors combined with mechanical signals, such as under stretching or compression modes, improved the proliferation and distribution of the seeded human heart cells throughout the scaffold volume and further stimulated the formation and organization of ECM; all of which attributed to the improvement in the mechanical strength of the cardiac graft (Akhyari et al., 2002; Zimmermann et al., 2002a, 2002b). These encouraging achievements still face significant difficulties. Most bioreactors cannot supply enough nutrients and oxygen to a growing thick tissue. Whereas human heart muscle is about 1 cm thick, growth in a bioreactor typically stops once the tissue is about 100 μm, or less than 10 cell layers thick (Colton, 1995). Beyond this thickness, the innermost cells are too far from the supply of fresh growth medium to thrive. Future bioreactors for cardiac tissue engineering should combine both perfusion and mechanical stimuli, for example by allowing for adjustable pulsatile flow and varying levels of pressure. Such bioreactors are currently under development for engineering heart valves ex-vivo (Sodian et al., 2000; Dohmen et al., 2002).

### 4.3. Transplantation of engineered construct

In one of the earliest studies of cell therapy to repair the infarcted myocardium, Leor et al. (1996) implanted small (1–3 mm) chunks of fetal rat and human myocardial tissues in infarcted rat heart and showed that the implants survived for at least 2 months in the infarcted myocardium. Their findings raised hope that if we could engineered cardiac tissue in vitro, it can be used for myocardial tissue repair.

Li et al. (1999) reported that bioengineered cardiac grafts can be made of fetal cardiac cells and 3D gelatin mesh. The cells in the graft formed cardiac-like tissue and contracted spontaneously. However, after transplantation on infarcted myocardium of rat, compared with control, LV-developed pressure was lower in hearts into which either a cell seeded or unseeded graft had been implanted. The authors proposed that inappropriate sizing of the grafts interferes with the contractility of the viable myocardium (Li et al., 1999).

We have reported successful seeding of rat fetal cardiomyocytes into porous scaffolds composed of alginate sponges (Leor et al., 2000). We found that the seeded fetal cardiac cells retained viability within the scaffolds and within 24 hr formed multicellular beating cell clusters. Following implantation of the cellular constructs into the infarcted myocardium, some of the cells appeared to differentiate into mature myocardial fibers. The implanted cardiac grafts were supplied by intensive neovascularization, which evidently contributed to the survival of the cells in the grafts. The biografts attenuated LV dilatation and deterioration of heart function. The mechanism behind this beneficial effect was unclear. A direct contribution of the biograft to contractility is unlikely because only a relatively small fraction of the biograft was composed of myocardial tissue. Attenuation of infarct expansion by virtue of the elastic properties of bioartificial grafts is possible. Restraining the expansion of the LV by a mesh placed over the infarcted myocardium, preserves LV geometry and resting function in a sheep model of MI (Kelley et al., 1999) and is now tested in a clinical trial (Oz et al., 2003). Angiogenesis induced by growth factors secreted from the seeded cells, resulting in improved collateral flow and augmentation of contractility, is also a possible mechanism (Kellar et al., 2001).

Zimmermann et al. (2002a) created EHT by mixing cardiac myocytes from neonatal Fischer 344 rats with liquid collagen type I, Matrigel, and serum-containing culture medium. EHT were designed in circular shape to fit around the circumference of hearts from syngeneic rats. After 12 days in culture, EHT were implanted on uninjured hearts. Fourteen days after implantation, EHT were heavily vascularized and retained a well-organized heart muscle structure as indicated by immunolabeling of actinin, connexin 43, and cadherins. Ultrastructural analysis demonstrated that implanted EHT surpassed the degree of differentiation reached before implantation. Contractile function of EHT grafts was preserved in vivo but, compared with baseline values, did not improve LV function as indicated by serial echocardiography studies (Zimmermann et al., 2002a). In addition, the transplantation results were limited by immune response of the host animal against the biomaterial mixture and the need for a continuous immunosuppression (Zimmermann et al., 2002a).

In another study, Matsubayashi et al. (2003) showed that surgical repair with smooth muscle-cell seeded grafts reduced abnormal chamber distensibility and improved LV function after MI as compared with unseeded grafts. The authors proposed that bioengineered muscle grafts may be superior to synthetic materials for the surgical repair of LV scar (Matsubayashi et al., 2003).

With all the effort invested so far, the in vitro approach for cardiac tissue engineering followed by transplantation of the engineered tissue has shown limited success. After transplantation, rapid vascularization, adequate perfusion, cell survival, integration and function of the engineered cardiac patch remain critical steps in the translation of in vitro achievements into effective therapeutic tool (Zandonella, 2003; Leor & Cohen, 2004).

### 4.4. Repair of congenital heart defects

The achievements in the field of myocardial tissue engineering could be used for the repair of congenital cardiac defects. The synthetic materials currently available for the repair of cardiac defects are nonviable, do not grow as the child develops, and do not contract synchronously with the heart. Sakai et al. (2001) created a beating patch by seeding fetal cardiomyocytes in a biodegradable gelatin scaffold in vitro and used it to replace the right ventricular outflow tract (RVOT) in syngeneic rats. The cells survived in the RVOT after the scaffold dissolved 12 weeks after implantation. In addition, the unseeded patches encouraged the ingrowth of fibrous tissue as the scaffold dissolved and the patches remained completely endothelialized. However, a significant inflammatory reaction was noted in the patch at 4 weeks as the scaffold dissolved (Sakai et al., 2001). In subsequent studies, they have proposed that autologous smooth muscle cell (SMC)-seeded biodegradable scaffolds could be a suitable construct to repair RVOT cardiac anomalies (Ozawa et al., 2002, 2004). In another study, Krupnick et al. (2002) demonstrated full replacement of the ventricular free wall of the heart by scaffold made of polytetrafluoroethylene, polylactide mesh, and type-I and -IV collagen hydrogel that was seeded with mesenchymal stem cells.

## 5. Construct vascularization

### 5.1. Angiogenesis and neovascularization

The 3D cell constructs that are developed ex vivo usually lack the vascular network that exists in normal

tissues. One of the most important requirements from a tissue engineering scaffold is its ability to support vascular infiltration (Patel & Mikos, 2004). After implantation, the engineered heart muscle should survive the ischemic period until new vessels invade the graft to maintain viability and function. Implanted cardiomyocytes are very sensitive to prolong ischemia and may die by necrosis and apoptosis. Thus, to become clinically relevant, a myocardial tissue engineered graft requires persistent neovascularization, or angiogenesis, for its growth and survival.

The extent of angiogenesis is determined by the regulating molecules that grafted cells and host cells release into the microenvironment of the engineered tissue. Angiogenesis can occur through either sprouting or nonsprouting processes. Sprouting angiogenesis involves the branching of new capillaries from preexisting vessels. Nonsprouting angiogenesis results from the hematopoietic precursor cells can be incorporated into the growing vascular bed (Losordo & Dimmeler, 2004a, 2004b).

Recent advances in our understanding of the process of blood vessel growth have provided significant tools for the neovascularization of bioengineered tissues. Several growth factors serve as stimuli for endothelial cell proliferation and migration as well as the formation of new blood vessels. Vascular epithelial growth factor (VEGF) is a major regulator of neovascularization. VEGF plays a major role in the early development of blood-cell progenitors (Losordo & Dimmeler, 2004a, 2004b). Basic fibroblast growth factor (bFGF) is a potent inducer of endothelial cell proliferation and blood-vessel growth in vitro and in vivo. VEGF and bFGF have been injected into under-vascularized ischemic myocardial tissues, resulting in new blood-vessel formation and tissue perfusion (Losordo & Dimmeler, 2004a, 2004b).

### 5.2. Local delivery of growth factors

Site-specific delivery of angiogenic growth factors from tissue-engineered devices should provide an efficient means of stimulating localized vessel recruitment to the cell transplants and would enhance cell survival and function. Local growth factors delivery will avoid serious adverse effects such as hyper permeability, edema, hypotension, and accelerated atherosclerosis (Epstein et al., 2001).

Angiogenic factors have been incorporated into bioengineered tissues and have facilitated blood-vessel growth (Peters et al., 1998; Richardson et al., 2001; Perets et al., 2003). For example, Perets et al. (2003) described the construction of a novel porous alginate scaffold that incorporates tiny poly (lactic-*co*-glycolic acid) microspheres capable of controlling the release of angiogenic factors, such as bFGF. In vitro, bFGF was released from the porous composite scaffolds in a controlled manner and it was biologically active as assessed by its ability to induce the proliferation of cardiac fibroblasts. The controlled delivery of bFGF from the three-dimensional scaffolds accelerates scaffold vascularization after implantation on the mesenteric membrane in rat peritoneum. The released bFGF induced the formation of large and matured blood vessels (Perets et al., 2003). Implantation of such bFGF impregnated alginate scaffold on the infarcted myocardium of rat stimulate angiogenesis in both the infarcted myocardium and the biograft (Fig. 2).

This strategy can improve survival of engrafted cells. Lee et al. (2002) showed that the angiogenic growth factor bFGF can be incorporated into degradable polymers used as delivery devices for hepatocyte transplantation. Implantation of these devices increases angiogenesis into the device and increases hepatocyte engraftment.

Richardson et al. (2001) moved one step forward by creating a new polymeric system that deliver two or more growth factors, with controlled dose and rate of delivery. The utility of this system was investigated in the context of therapeutic angiogenesis. They showed that dual delivery of VEGF-165 and platelet-derived growth factor (PDGF)-BB, each with distinct kinetics, from a single, structural polymer scaffold results in the rapid formation of a mature vascular network (Richardson et al., 2001).

Other approaches such as pre-vascularization of the implanted scaffold prior to cell seeding (Hench et al., 2004; Wu et al., 2004) and incorporation of endothelial cells into the bioengineered tissues have produced encouraging results (Park et al., 1999; Narmoneva et al., 2004) and could be applied to myocardial tissue engineering. Preimplantation of endothelial cells promote myocyte survival and

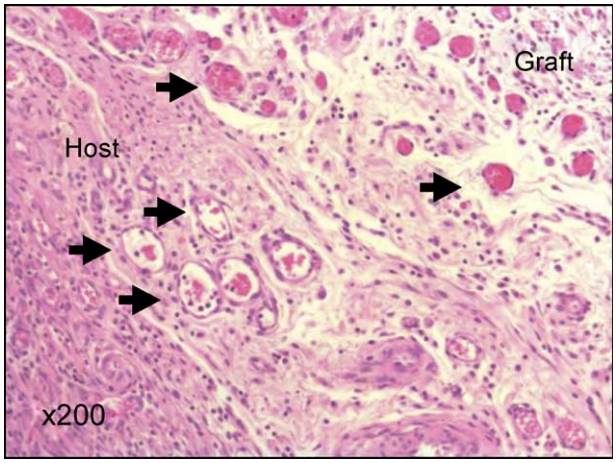


Fig. 2. Micrograph of alginate scaffold impregnated with basic fibroblast growth factor at 1 month after implantation into the infarcted myocardium of rat. Microspheres can be incorporated throughout the scaffold by mixing them into the alginate scaffold. The microspheres accelerate functional blood vessel formation both in the biograft and the host myocardium (arrows).

enhance spatial organization in 3D configuration (Narmoneva et al., 2004).

## 6. In situ regeneration

### 6.1. In situ tissue engineering

While in vitro tissue engineering to create engineered muscle construct in bioreactor is fascinating and exciting, it faces significant difficulties, in particular constructing significant cardiac muscle from scaffold and cells in vitro, and poor graft survival. Thus, our group is now testing the in situ tissue engineering approach. In this approach, unseeded alginate scaffolds are implanted on the damaged myocardium and after their vascularization, they create a friendly environment and space for the implanted cardiomyocytes. To accelerate angiogenesis and engraftment, the implanted scaffold could be impregnated with bioactive molecules that improve viability and survival and may enhance stem cell homing and self-repair. There is now accumulating evidence that the heart contains resident progenitor cells that can be induced to develop into cardiac muscle and vascular tissue (Beltrami et al., 2003; Oh et al., 2003; Matsuura et al., 2004). These cardiac progenitors could be recruited to repair the infarcted myocardium. An important advantage of this concept of in situ tissue engineering is the feasibility to employ it with catheter-based approach and to avoid the need for surgical thoracotomy.

### 6.2. In situ regeneration by bioactive materials

With this approach, the biomaterial itself or its degradation/dissolution products are used to stimulate local tissue repair. Bioactive materials release chemicals in the form of ionic dissolution products, or growth factors such as bone morphogenic protein (BMP), at controlled rates, by diffusion or network breakdown, that activate the cells in contact with the stimuli. The cells produce additional growth factors that in turn stimulate multiple generations of growing cells to self-assemble into tissues in situ along the biochemical and biomechanical gradients that are present. These materials, once implanted, will help the body heal itself (Hench & Polak, 2002).

Molecular modifications of the biomaterial intend to elicit specific interactions with cell integrins and thereby direct cell proliferation, differentiation, and ECM production and organization. The mechanism for in situ tissue regeneration involves up-regulation of genes that control the cell cycle, mitosis, and differentiation. Gene activation by controlled ion release provides the conceptual basis for molecular design of a third generation of biomaterials optimized for in situ tissue regeneration (Hench et al., 2004).

### 6.3. In situ regeneration by bioactive molecules

In situ regeneration in the injured myocardium can be stimulated by direct delivery of several cytokines that potentially stimulate myocardial healing and repair in the setting of MI (Nian et al., 2004). Those cytokines may induce recruitment of bone-marrow-derived primitive stem cells in the healing infarct, which may differentiate into endothelial cells and even lead to myocardial regeneration. These molecules include cytokines such as granulocyte colony-stimulating factor (G-CSF; Takano et al., 2003; Minatoguchi et al., 2004), stromal derived growth factor (SDF-1; Hiasa et al., 2004), leukemia inhibitory factor (LIF; Zou et al., 2003), insulin-like growth factor (IGF-1; Musaro et al., 2004), and erythropoietin (EPO; Calvillo et al., 2003). In addition to their role in expanding and mobilizing bone marrow-derived progenitor cells, they may have direct myocardial-protective effects.

G-CSF plays a critical role in regulation of proliferation, differentiation, and survival of myeloid progenitor cells. G-CSF also causes a marked increase in the release of hematopoietic stem cells into the peripheral blood circulation, a process termed mobilization. Recently, G-CSF has been reported to stimulate healing and repair (Minatoguchi et al., 2004), to improve cardiac function, and to reduce mortality after acute MI (Orlic et al., 2001; Ohtsuka et al., 2004). Although the mechanism by which G-CSF ameliorates cardiac dysfunction is not fully understood, there is the possibility that G-CSF may regenerate cardiac myocytes and blood vessels through mobilization of bone marrow stem cells (Orlic et al., 2001).

EPO is important for erythrocyte survival and differentiation; it has the ability to maintain vascular autoregulation and attenuating primary (apoptotic) and secondary (inflammatory) causes of cell death. In a rat model of infarction, EPO reduces cardiac myocyte loss by ≈50%, sufficient to normalize hemodynamic function after reperfusion (Calvillo et al., 2003). The hematopoietic growth factor EPO has been found to mediate repair and regeneration after brain and spinal cord injury, including the recruitment of stem cells into the region of damage (Heeschen et al., 2003).

SDF-1 is a chemokine considered to play an important role in the trafficking and survival of hematopoietic, endothelial progenitors and mesenchymal stem cells. Locally delivered SDF-1 augments vasculogenesis and subsequently contributes to ischemic neovascularization in vivo by augmenting endothelial progenitor cell (EPC) recruitment in ischemic tissues (Askari et al., 2003; Yamaguchi et al., 2003; Hiasa et al., 2004). Finally, IGF-1 is a cytokine that can enhances nuclear phospho-Akt and telomerase and delaying cardiomyocyte aging and death (Li et al., 1997; Musaro et al., 2004; Torella et al., 2004). It can improve stem cell homing, healing, and regeneration of the injured muscle (Winn et al., 2002). Taken together,

cytokine-mediated regenerative therapy may evolve to be a novel therapeutic strategy for MI.

### 6.4. Injectable tissue engineering

Most of the efforts in cardiac tissue engineering center on the use of implantable scaffolds that deliver cells to the epicardial surface. However, many strategies of cell or gene delivery to repair the infarcted myocardium are moving toward catheter-based approach. This semi-invasive approach avoids the risk of open chest surgery and anesthetic and is favored by both patients and physicians. The injectable scaffold facilitates repair after infarction by providing a matrix support within which cells are retained, migrate, and neoangiogenesis takes place.

Christman et al. (2004a) examined the effects of fibrin glue as an injectable scaffold. The bioactive fibrin scaffold is also known to be angiogenic (Bootle-Wilbraham et al., 2001; van Hinsbergh et al., 2001). They subjected rats to short (17 min) coronary artery occlusion followed by reperfusion. Ten days after MI, either 0.5% BSA in phosphate-buffered saline (PBS), fibrin glue alone, skeletal myoblasts alone, or skeletal myoblasts in fibrin glue were injected into the ischemic LV. Echocardiography studies showed that fibrin glue preserved infarct wall thickness and cardiac function after MI (Christman et al., 2004a). In a subsequent study, this group showed that fibrin glue improves myoblast graft retention and survival, reduces infarct expansion, and induces neovascularization in the infarcted myocardium (Christman et al., 2004b). These findings were supported by Ryu et al. (2005) who showed that implantation of bone marrow mononuclear cells using injectable fibrin matrix further enhances neovascularization in infarcted myocardium compared to cell implantation without matrix. More recently, Kofidis et al. (2004) have shown that seeding mouse embryonic stem cells in a liquid Matrigel can create a “Liquid bioartificial tissue”. Injection of the Matrigel-based liquid cell mixture into the infarcted myocardium in a Lewis rat heterotopic heart transplant model restored injured myocardium without distorting its geometry. Taken together, these works suggest that injectable biomaterials can serve as a cell implantation matrix that enhances neovascularization and repair of the infarcted myocardium.

We have recently presented preliminary data that show that injection of biodegradable alginate solution into the infarcted myocardium stimulates neoangiogenesis and efficiently attenuates infarct expansion, heart dilatation, and dysfunction (Leor et al., 2004). Our preliminary work enables minimally invasive, catheter-based, acellular option to facilitate neovascularization, self-repair, and rejuvenation of the infarcted myocardium. The injectable bioactive material proposes a viable solution to the difficulties in achieving appropriate cells to treat MI and future strategy of catheter-based injectable tissue engineering (Cohen & Leor, 2004).

## 7. Challenges, risks, and future perspectives

The ability to engineer or regenerate lost myocardial tissue due to injury, aging, disease, or genetic abnormality holds great promise. However, the area of myocardial tissue engineering still faces significant difficulties and challenges (Table 3). One of the challenges is the design of bioactive scaffolds, which allow composition variation to accommodate divergence in the evolving myocardial structure. There is a need for development of strategies to promote vascularization and/or innervations within engineered myocardial tissue. Consideration should be made for multiple design and delivery approaches for in vitro myocardial tissue preparations. Other important goals are to increase our understanding of the basic principles governing myocardial tissue formation, function, and failure, including the assembly of multiple cell types and biomaterials into multi-dimensional structures that mimic the architecture and function of native heart muscle.

In addition to laboratory grown myocardial construct, more research is warranted in the area of regenerating functional myocardium in situ: There is increasing evidence that heart may be capable of self-regeneration (Anversa et al., 2004). Future studies are needed to assess the functional significance of these changes in heart muscle, and the potential of other agents to control regeneration of myocardial tissue. If successful, these strategies will solve the problem of organ donor shortage and can be used for surgical repair of the infarcted myocardium or congenital cardiac defects. The ability to provide the tools for replacing damaged myocardium will have a dramatic impact on cardiovascular medicine. However, the success of this

Table 3
Major challenges and risks in the application of myocardial tissue engineering

| |
|---|
| • Cell |
| Best source |
| Expansion |
| Control of differentiation |
| Cell survival |
| Integration |
| Non-immunogenic |
| • Scaffold |
| Non-toxic |
| Non-immunogenic |
| Biodegradable |
| Bioactive |
| Flexible |
| Integration |
| • Mode of delivery |
| • Vascularization |
| • Infection |

exciting concept depends on carefully designed experiments to achieve this ambitious goal.

## References


Akhyari, P., Fedak, P. W., Weisel, R. D., Lee, T. Y., Verma, S., Mickle, D. A., et al. (2002). Mechanical stretch regimen enhances the formation of bioengineered autologous cardiac muscle grafts. *Circulation 106*, I137–I142.

Akins, R. E., Boyce, R. A., Madonna, M. L., Schroedl, N. A., Gonda, S. R., McLaughlin, T. A., et al. (1999). Cardiac organogenesis in vitro: reestablishment of three-dimensional tissue architecture by dissociated neonatal rat ventricular cells. *Tissue Eng 5*, 103–118.

Anversa, P., Sussman, M. A., & Bolli, R. (2004). Molecular genetic advances in cardiovascular medicine: focus on the myocyte. *Circulation 109*, 2832–2838.

Askari, A. T., Unzek, S., Popovic, Z. B., Goldman, C. K., Forudi, F., Kiedrowski, M., et al. (2003). Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet 362*, 697–703.

Beltrami, A. P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., et al. (2003). Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell 114*, 763–776.

Bootle-Wilbraham, C. A., Tazzyman, S., Thompson, W. D., Stirk, C. M., & Lewis, C. E. (2001). Fibrin fragment E stimulates the proliferation, migration and differentiation of human microvascular endothelial cells in vitro. *Angiogenesis 4*, 269–275.

Bursac, N., Papadaki, M., Cohen, R. J., Schoen, F. J., Eisenberg, S. R., Carrier, R., et al. (1999). Cardiac muscle tissue engineering: Ttoward an in vitro model for electrophysiological studies. *Am J Physiol 277*, H433–H444.

Bursac, N., Papadaki, M., White, J. A., Eisenberg, S. R., Vunjak-Novakovic, G., & Freed, L. E. (2003). Cultivation in rotating bioreactors promotes maintenance of cardiac myocyte electrophysiology and molecular properties. *Tissue Eng 9*, 1243–1253.

Calvillo, L., Latini, R., Kajstura, J., Leri, A., Anversa, P., Ghezzi, P., et al. (2003). Recombinant human erythropoietin protects the myocardium from ischemia-reperfusion injury and promotes beneficial remodeling. *Proc Natl Acad Sci U S A 100*, 4802–4806.

Carrier, R. L., Papadaki, M., Rupnick, M., Schoen, F. J., Bursac, N., Langer, R., et al. (1999). Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. *Biotechnol Bioeng 64*, 580–589.

Carrier, R. L., Rupnick, M., Langer, R., Schoen, F. J., Freed, L. E., & Vunjak-Novakovic, G. (2002). Perfusion improves tissue architecture of engineered cardiac muscle. *Tissue Eng 8*, 175–188.

Christman, K. L., Fok, H. H., Sievers, R. E., Fang, Q., & Lee, R. J. (2004a). Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. *Tissue Eng 10*, 403–409.

Christman, K. L., Vardanian, A. J., Fang, Q., Sievers, R. E., Fok, H. H., & Lee, R. J. (2004b). Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. *J Am Coll Cardiol 44*, 654–660.

Cohen, S., & Leor, J. (2004). Rebuilding broken hearts. *Sci Am 291*, 44–51.

Colton, C. K. (1995). Implantable biohybrid artificial organs. *Cell Transplant 4*, 415–436.

Copeland, J. G., Smith, R. G., Arabia, F. A., Nolan, P. E., Sethi, G. K., Tsau, P. H., et al. (2004). Cardiac replacement with a total artificial heart as a bridge to transplantation. *N Engl J Med 351*, 859–867.

Dar, A., Shachar, M., Leor, J., & Cohen, S. (2002). Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds. *Biotechnol Bioeng 80*, 305–312.

Dimmeler, S., & Vasa-Nicotera, M. (2003). Aging of progenitor cells: Limitation for regenerative capacity? *J Am Coll Cardiol 42*, 2081–2082.

Dohmen, P. M., Ozaki, S., Verbeken, E., Yperman, J., Flameng, W., & Konertz, W. F. (2002). Tissue engineering of an auto-xenograft pulmonary heart valve. *Asian Cardiovasc Thorac Ann 10*, 25–30.

Epstein, S. E., Kornowski, R., Fuchs, S., & Dvorak, H. F. (2001). Angiogenesis therapy: Amidst the hype, the neglected potential for serious side effects. *Circulation 104*, 115–119.

Etzion, S., Kedes, L. H., Kloner, R. A., & Leor, J. (2001). Myocardial regeneration: Present and future trends. *Am J Cardiovasc Drugs 1*, 233–244.

Griffith, L. G., & Lopina, S. (1998). Microdistribution of substratum-bound ligands affects cell function: Hepatocyte spreading on PEO-tethered galactose. *Biomaterials 19*, 979–986.

Halstenberg, S., Panitch, A., Rizzi, S., Hall, H., & Hubbell, J. A. (2002). Biologically engineered protein-graft-poly(ethylene glycol) hydrogels: a cell adhesive and plasmin-degradable biosynthetic material for tissue repair. *Biomacromolecules 3*, 710–723.

Heeschen, C., Aicher, A., Lehmann, R., Fichtlscherer, S., Vasa, M., Urbich, C., et al. (2003). Erythropoietin is a potent physiological stimulus for endothelial progenitor cell mobilization. *Blood 102*, 1340–1346.

Hench, L. L., & Polak, J. M. (2002). Third-generation biomedical materials. *Science 295*, 1014–1017.

Hench, L. L., Xynos, I. D., & Polak, J. M. (2004). Bioactive glasses for in situ tissue regeneration. *J Biomater Sci Polym Ed 15*, 543–562.

Hiasa, K. -i., Ishibashi, M., Ohtani, K., Inoue, S., Zhao, Q., Kitamoto, S., et al. (2004). Gene transfer of stromal cell-derived factor-1{alpha} enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: Next-generation chemokine therapy for therapeutic neovascularization. *Circulation 109*, 2454–2461.

Humphries, M. J., Akiyama, S. K., Komoriya, A., Olden, K., & Yamada, K. M. (1986). Identification of an alternatively spliced site in human plasma fibronectin that mediates cell type-specific adhesion. *J Cell Biol 103*, 2637–2647.

Jessup, M., & Brozena, S. (2003). Heart failure. *N Engl J Med 348*, 2007–2018.

Kadner, A., Zund, G., Maurus, C., Breymann, C., Yakarisik, S., Kadner, G., et al. (2004). Human umbilical cord cells for cardiovascular tissue engineering: A comparative study. *Eur J Cardiothorac Surg 25*, 635–641.

Kamelger, F. S., Marksteiner, R., Margreiter, E., Klima, G., Wechselberger, G., Hering, S., et al. (2004). A comparative study of three different biomaterials in the engineering of skeletal muscle using a rat animal model. *Biomaterials 25*, 1649–1655.

Kellar, R. S., Landeen, L. K., Shepherd, B. R., Naughton, G. K., Ratcliffe, A., & Williams, S. K. (2001). Scaffold-based three-dimensional human fibroblast culture provides a structural matrix that supports angiogenesis in infarcted heart tissue. *Circulation 104*, 2063–2068.

Kelley, S. T., Malekan, R., Gorman III, J. H., Jackson, B. M., Gorman, R. C., Suzuki, Y., et al. (1999). Restraining infarct expansion preserves left ventricular geometry and function after acute anteroapical infarction. *Circulation 99*, 135–142.

Kim, M. R., Jeong, J. H., & Park, T. G. (2002). Swelling induced detachment of chondrocytes using RGD-modified poly(*N*-isopropylacrylamide) hydrogel beads. *Biotechnol Prog 18*, 495–500.

Kofidis, T., Akhyari, P., Boublik, J., Theodorou, P., Martin, U., Ruhparwar, A., et al. (2002). In vitro engineering of heart muscle: Artificial myocardial tissue. *J Thorac Cardiovasc Surg 124*, 63–69.

Kofidis, T., Lenz, A., Boublik, J., Akhyari, P., Wachsmann, B., Mueller-Stahl, K., et al. (2003). Pulsatile perfusion and cardiomyocyte viability in a solid three-dimensional matrix. *Biomaterials 24*, 5009–5014.

Kofidis, T., De Bruin, J. L., Hoyt, G., Lebl, D. R., Tanaka, M., Yamane, T., et al. (2004). Injectable bioartificial myocardial tissue for large-scale intramural cell transfer and functional recovery of injured heart muscle. *J Thorac Cardiovasc Surg 128*, 571–578.

Koo, L. Y., Irvine, D. J., Mayes, A. M., Lauffenburger, D. A., & Griffith, L. G. (2002). Co-regulation of cell adhesion by nanoscale RGD organization and mechanical stimulus. *J Cell Sci 115*, 1423–1433.

Krupnick, A. S., Kreisel, D., Szeto, W. Y., Popma, S. H., & Rosengard, B. R. (2001). A murine model of left ventricular tissue engineering. *J Heart Lung Transplant 20*, 197–198.

Krupnick, A. S., Kreisel, D., Engels, F. H., Szeto, W. Y., Plappert, T., Popma, S. H., et al. (2002). A novel small animal model of left ventricular tissue engineering. *J Heart Lung Transplant 21*, 233–243.

Langer, R., & Tirrell, D. A. (2004). Designing materials for biology and medicine. *Nature 428*, 487–492.

Lanza, R., Moore, M. A., Wakayama, T., Perry, A. C., Shieh, J. H., Hendrikx, J., et al. (2004). Regeneration of the infarcted heart with stem cells derived by nuclear transplantation. *Circ Res 94*, 820–827.

Lee, M. S., & Makkar, R. R. (2004). Stem-cell transplantation in myocardial infarction: A status report. *Ann Intern Med 140*, 729–737.

Lee, H., Cusick, R. A., Browne, F., Ho Kim, T., Ma, P. X., Utsunomiya, H., et al. (2002). Local delivery of basic fibroblast growth factor increases both angiogenesis and engraftment of hepatocytes in tissue-engineered polymer devices. *Transplantation 73*, 1589–1593.

Leor, J., & Cohen, S. (2004). Myocardial tissue engineering: creating a muscle patch for a wounded heart. *Ann NY Acad Sci 1015*, 312–319.

Leor, J., Patterson, M., Quinones, M. J., Kedes, L. H., & Kloner, R. A. (1996). Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation 94*, II332–II336.

Leor, J., Aboulafia-Etzion, S., Dar, A., Shapiro, L., Barbash, I. M., Battler, A., et al. (2000). Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium? *Circulation 102*, III56–III61.

Leor, J., Miller, L., Feinberg, M. S., Shachar, S., Landa, N., Holbova, R., et al. (2004). A novel injectable alginate scaffold promotes angiogenesis and preserves left ventricular geometry and function after extensive myocardial infarction in rat (abstract). *Circulation 110*(Suppl.), III279.

Levenberg, S., Huang, N. F., Lavik, E., Rogers, A. B., Itskovitz-Eldor, J., & Langer, R. (2003). Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. *Proc Natl Acad Sci U S A 100*, 12741–12746.

Li, R. K. (2004). Cell transplantation to improve heart function: cell or matrix. *Yonsei Med J 45*(Suppl.), S72–S73.

Li, Q., Li, B., Wang, X., Leri, A., Jana, K. P., Liu, Y., et al. (1997). Overexpression of insulin-like growth factor-1 in mice protects from myocyte death after infarction, attenuating ventricular dilation, wall stress, and cardiac hypertrophy. *J Clin Invest 100*, 1991–1999.

Li, R. K., Jia, Z. Q., Weisel, R. D., Mickle, D. A., Choi, A., & Yau, T. M. (1999). Survival and function of bioengineered cardiac grafts. *Circulation 100*, II63–II69.

Li, R. K., Yau, T. M., Weisel, R. D., Mickle, D. A., Sakai, T., Choi, A., et al. (2000). Construction of a bioengineered cardiac graft. *J Thorac Cardiovasc Surg 119*, 368–375.

Losordo, D. W., & Dimmeler, S. (2004a). Therapeutic angiogenesis and vasculogenesis for ischemic disease: Part I. Angiogenic cytokines. *Circulation 109*, 2487–2491.

Losordo, D. W., & Dimmeler, S. (2004b). Therapeutic angiogenesis and vasculogenesis for ischemic disease: Part II. Cell-based therapies. *Circulation 109*, 2692–2697.

Mann, B. K., & West, J. L. (2002). Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds. *J Biomed Mater Res 60*, 86–93.

Matsubayashi, K., Fedak, P. W., Mickle, D. A., Weisel, R. D., Ozawa, T., & Li, R. K. (2003). Improved left ventricular aneurysm repair with bioengineered vascular smooth muscle grafts. *Circulation 108*(Suppl. 1), II219–II225.

Matsuura, K., Nagai, T., Nishigaki, N., Oyama, T., Nishi, J., Wada, H., et al. (2004). Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem 279*, 11384–11391.

McDevitt, T. C., Angello, J. C., Whitney, M. L., Reinecke, H., Hauschka, S. D., Murry, C. E., et al. (2002). In vitro generation of differentiated cardiac myofibers on micropatterned laminin surfaces. *J Biomed Mater Res 60*, 472–479.

McDevitt, T. C., Woodhouse, K. A., Hauschka, S. D., Murry, C. E., & Stayton, P. S. (2003). Spatially organized layers of cardiomyocytes on biodegradable polyurethane films for myocardial repair. *J Biomed Mater Res 66A*, 586–595.

Minatoguchi, S., Takemura, G., Chen, X. H., Wang, N., Uno, Y., Koda, M., et al. (2004). Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by postinfarction granulocyte colony-stimulating factor treatment. *Circulation 109*, 2572–2580.

Mironov, V., Boland, T., Trusk, T., Forgacs, G., & Markwald, R. R. (2003). Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol 21*, 157–161.

Mummery, C., Ward-Van Oostwaard, D., Doevendans, P., Spijker, R., Van Den Brink, S., Hassink, R., et al. (2003). Differentiation of human embryonic stem cells to cardiomyocytes: Role of coculture with visceral endoderm-like cells. *Circulation 107*, 2733–2740.

Musaro, A., Giacinti, C., Borsellino, G., Dobrowolny, G., Pelosi, L., Cairns, L., et al. (2004). Stem cell-mediated muscle regeneration is enhanced by local isoform of insulin-like growth factor 1. *Proc Natl Acad Sci USA 101*, 1206–1210.

Narmoneva, D. A., Vukmirovic, R., Davis, M. E., Kamm, R. D., & Lee, R. T. (2004). Endothelial cells promote cardiac myocyte survival and spatial reorganization. Implications for cardiac regeneration. *Circulation*, (01.CIR.0000140667.0000137070.0000140607).

Nguyen, H., Qian, J. J., Bhatnagar, R. S., & Li, S. (2003). Enhanced cell attachment and osteoblastic activity by P-15 peptide-coated matrix in hydrogels. *Biochem Biophys Res Commun 311*, 179–186.

Nian, M., Lee, P., Khaper, N., & Liu, P. (2004). Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res 94*, 1543–1553.

Oh, H., Bradfute, S. B., Gallardo, T. D., Nakamura, T., Gaussin, V., Mishina, Y., et al. (2003). Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A 100*, 12313–12318.

Ohtsuka, M., Takano, H., Zou, Y., Toko, H., Akazawa, H., Qin, Y., et al. (2004). Cytokine therapy prevents left ventricular remodeling and dysfunction after myocardial infarction through neovascularization. *FASEB J 18*, 851–853.

Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., et al. (2001). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A 98*, 10344–10349.

Oz, M. C., Konertz, W. F., Kleber, F. X., Mohr, F. W., Gummert, J. F., Ostermeyer, J., et al. (2003). Global surgical experience with the Acorn cardiac support device. *J Thorac Cardiovasc Surg 126*, 983–991.

Ozawa, T., Mickle, D. A., Weisel, R. D., Koyama, N., Ozawa, S., & Li, R. K. (2002). Optimal biomaterial for creation of autologous cardiac grafts. *Circulation 106*, I176–I182.

Ozawa, T., Mickle, D. A., Weisel, R. D., Matsubayashi, K., Fujii, T., Fedak, P. W., et al. (2004). Tissue-engineered grafts matured in the right ventricular outflow tract. *Cell Transplant 13*, 169–177.

Papadaki, M., Bursac, N., Langer, R., Merok, J., Vunjak-Novakovic, G., & Freed, L. E. (2001a). Tissue engineering of functional cardiac muscle: Molecular, structural, and electrophysiological studies. *Am J Physiol Heart Circ Physiol 280*, H168–H178.

Papadaki, M., Bursac, N., Langer, R., Merok, J., Vunjak-Novakovic, G., & Freed, L. E. (2001b). Tissue engineering of functional cardiac muscle: Molecular, structural, and electrophysiological studies. *Am J Physiol Heart Circ Physiol 280*, H168–H178.

Park, H. J., Yoo, J. J., Kershen, R. T., Moreland, R., & Atala, A. (1999). Reconstitution of human corporal smooth muscle and endothelial cells in vivo. *J Urol 162*, 1106–1109.

Pasumarthi, K. B., & Field, L. J. (2002). Cardiomyocyte cell cycle regulation. *Circ Res 90*, 1044–1054.

Patel, Z. S., & Mikos, A. G. (2004). Angiogenesis with biomaterial-based drug- and cell-delivery systems. *J Biomater Sci Polym Ed 15*, 701–726.

Perets, A., Baruch, Y., Weisbuch, F., Shoshany, G., Neufeld, G., & Cohen, S. (2003). Enhancing the vascularization of three-dimensional

porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. *J Biomed Mater Res 65A*, 489–497.

Peters, M. C., Isenberg, B. C., Rowley, J. A., & Mooney, D. J. (1998). Release from alginate enhances the biological activity of vascular endothelial growth factor. *J Biomater Sci Polym Ed 9*, 1267–1278.

Pratt, A. B., Weber, F. E., Schmoekel, H. G., Muller, R., & Hubbell, J. A. (2004). Synthetic extracellular matrices for in situ tissue engineering. *Biotechnol Bioeng 86*, 27–36.

Rauscher, F. M., Goldschmidt-Clermont, P. J., Davis, B. H., Wang, T., Gregg, D., Ramaswami, P., et al. (2003). Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation 108*, 457–463.

Richardson, T. P., Peters, M. C., Ennett, A. B., & Mooney, D. J. (2001). Polymeric system for dual growth factor delivery. *Nat Biotechnol 19*, 1029–1034.

Rowley, J. A., Madlambayan, G., & Mooney, D. J. (1999). Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials 20*, 45–53.

Ryu, J. H., Kim, I. K., Cho, S. W., Cho, M. C., Hwang, K. K., Piao, H., et al. (2005). Implantation of bone marrow mononuclear cells using injectable fibrin matrix enhances neovascularization in infarcted myocardium. *Biomaterials 26*, 319–326.

Sakai, T., Li, R. K., Weisel, R. D., Mickle, D. A., Kim, E. T., Jia, Z. Q., et al. (2001). The fate of a tissue-engineered cardiac graft in the right ventricular outflow tract of the rat. *J Thorac Cardiovasc Surg 121*, 932–942.

Scheubel, R. J., Zorn, H., Silber, R. E., Kuss, O., Morawietz, H., Holtz, J., et al. (2003). Age-dependent depression in circulating endothelial progenitor cells inpatients undergoing coronary artery bypass grafting. *J Am Coll Cardiol 42*, 2073–2080.

Shimizu, T., Yamato, M., Akutsu, T., Shibata, T., Isoi, Y., Kikuchi, A., et al. (2002a). Electrically communicating three-dimensional cardiac tissue mimic fabricated by layered cultured cardiomyocyte sheets. *J Biomed Mater Res 60*, 110–117.

Shimizu, T., Yamato, M., Isoi, Y., Akutsu, T., Setomaru, T., Abe, K., et al. (2002b). Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ Res 90*, e40.

Smits, P. C., van Geuns, R. J., Poldermans, D., Bountioukos, M., Onderwater, E. E., Lee, C. H., et al. (2003). Catheter-Based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure. Clinical experience with six-month follow-up. *J Am Coll Cardiol 42*, 2063–2069.

Sodian, R., Sperling, J. S., Martin, D. P., Egozy, A., Stock, U., Mayer Jr., J. E., et al. (2000). Fabrication of a trileaflet heart valve scaffold from a polyhydroxyalkanoate biopolyester for use in tissue engineering. *Tissue Eng 6*, 183–188.

Sun, Y., Kiani, M. F., Postlethwaite, A. E., & Weber, K. T. (2002). Infarct scar as living tissue. *Basic Res Cardiol 97*, 343–347.

Takahashi, T., Lord, B., Schulze, P. C., Fryer, R. M., Sarang, S. S., Gullans, S. R., et al. (2003). Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes. *Circulation 107*, 1912–1916.

Takano, H., Ohtsuka, M., Akazawa, H., Toko, H., Harada, M., Hasegawa, H., et al. (2003). Pleiotropic effects of cytokines on acute myocardial infarction: G-CSF as a novel therapy for acute myocardial infarction. *Curr Pharm Des 9*, 1121–1127.

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science 282*, 1145–1147.

Tiwari, A., Salacinski, H. J., Punshon, G., Hamilton, G., & Seifalian, A. M. (2002). Development of a hybrid cardiovascular graft using a tissue engineering approach. *FASEB J 16*, 791–796.

Torella, D., Rota, M., Nurzynska, D., Musso, E., Monsen, A., Shiraishi, I., et al. (2004). Cardiac stem cell and myocyte aging, heart failure, and insulin-like growth factor-1 overexpression. *Circ Res 94*, 514–524.

Vacanti, J. P., & Langer, R. (1999). Tissue engineering: The design and fabrication of living replacement devices for surgical reconstruction and transplantation. *Lancet 354*(Suppl. 1), SI32–S34.

van Hinsbergh, V. W., Collen, A., & Koolwijk, P. (2001). Role of fibrin matrix in angiogenesis. *Ann NY Acad Sci 936*, 426–437.

Vulliet, P. R., Greeley, M., Halloran, S. M., MacDonald, K. A., & Kittleson, M. D. (2004). Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet 363*, 783–784.

Winn, N., Paul, A., Musaro, A., & Rosenthal, N. (2002). Insulin-like growth factor isoforms in skeletal muscle aging, regeneration, and disease. *Cold Spring Harbor Symp Quant Biol 67*, 507–518.

Wu, X., Rabkin-Aikawa, E., Guleserian, K. J., Perry, T. E., Masuda, Y., Sutherland, F. W. H., et al. (2004). Tissue-engineered microvessels on three-dimensional biodegradable scaffolds using human endothelial progenitor cells. *Am J Physiol Heart Circ Physiol 287*, H480–H487.

Yamaguchi, J. -i., Kusano, K. F., Masuo, O., Kawamoto, A., Silver, M., Murasawa, S., et al. (2003). Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation 107*, 1322–1328.

Yoon, Y. -S., Park, J. -S., Tkebuchava, T., Luedeman, C., & Losordo, D. W. (2004). Unexpected severe calcification after transplantation of bone marrow cells in acute myocardial infarction. *Circulation* (01.CIR. 0000134696.0000108436.0000134665).

Zandonella, C. (2003). Tissue engineering: the beat goes on. *Nature 421*, 884–886.

Zimmermann, W. H., & Eschenhagen, T. (2003). Cardiac tissue engineering for replacement therapy. *Heart Fail Rev 8*, 259–269.

Zimmermann, W. H., Fink, C., Kralisch, D., Remmers, U., Weil, J., & Eschenhagen, T. (2000). Three-dimensional engineered heart tissue from neonatal rat cardiac myocytes. *Biotechnol Bioeng 68*, 106–114.

Zimmermann, W. H., Didie, M., Wasmeier, G. H., Nixdorff, U., Hess, A., Melnychenko, I., et al. (2002a). Cardiac grafting of engineered heart tissue in syngenic rats. *Circulation 106*, I151–I157.

Zimmermann, W. H., Schneiderbanger, K., Schubert, P., Didie, M., Munzel, F., Heubach, J. F., et al. (2002b). Tissue engineering of a differentiated cardiac muscle construct. *Circ Res 90*, 223–230.

Zimmermann, W. H., Melnychenko, I., & Eschenhagen, T. (2004). Engineered heart tissue for regeneration of diseased hearts. *Biomaterials 25*, 1639–1647.

Zou, Y., Takano, H., Mizukami, M., Akazawa, H., Qin, Y., Toko, H., et al. (2003). Leukemia inhibitory factor enhances survival of cardiomyocytes and induces regeneration of myocardium after myocardial infarction. *Circulation 108*, 748–753.